Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689235
USEPA DP Barcode: 435146
PMRA Data Code: 9.2.4.6
PMRA Study No. (UKID): 2627509
Data Requirement (Guideline): OECD Guidance Doc. No. 75

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.8%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

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Date: 15 February 2018

PMRA Reviewer: Vedad Izadi
Evaluation Officer, PMRA/EAD/ERSII
Date: 26 September 2017

Date Evaluation Completed: 26 September 2017

CITATION: Staffel J. 2015. Semi-field brood study to evaluate potential effects of BAS 440 00 I on the development of honeybee colonies (*Apis mellifera*). RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany. Report No. 737518. Sponsor: BASF SE. Report No. BASF Reg. Doc. #: 2015/1005007. USEPA MRID 49689235. PMRA UKID 2627509.

Executive Summary:

The semi-field (tunnel) study tested the effects of the afidopyropen formulated end-use product BAS 440 00 I (9.7% active ingredient) on honeybee (*Apis mellifera*) colonies with the intent of examining brood (*i.e.*, eggs, larvae, pupae) strength and colony strength (number and condition of adult bees/brood and available food reserves). The study design was based in part on OECD Guidance

Document No. 75. Nucleus bee colonies (containing 7,627 ± 544[1] adult bees/colony) within individual enclosures containing phacelia (*Phacelia tanacetifolia*) in full bloom were exposed, while bees were actively foraging, to either 50 g a.i./ha (0.04 lbs a.i./A) of BAS 440 00 I, a reference toxicant dimethoate at 480 g a.i./ha, or a water (negative) control treatment. Each treatment group consisted of four replicate tunnels, each containing a single nucleus colony; colonies were acclimated to the tunnels six days before applications. Colonies were maintained in the tunnels for 7 days after treatments (DAT, "exposure phase"), and then transferred to a remote monitoring site without a bee-attractive flowering crop for 34 days ("monitoring phase"). Adult and larval/pupal mortality were recorded from five days before, to 33 days after, treatments (-5 to 33 DAT); assessments included foraging activity (-5 to 7 DAT), colony condition (food stores, brood status, and colony strength) at -1, 4, 11, 22, 32, and 41 DAT. In addition to the four replicate tunnels in control and afidopyropen-treatment groups, there was an extra tunnel in each of these treatment groups used solely for residue monitoring.

The preliminary brood check indicated healthy colonies with all brood stages present, and a sufficient supply with nectar and pollen. Throughout the study, the number of food or brood cells did not differ statistically among the negative control, afidopyropen-treated, and dimethoate-treated groups. Treatment rates were not confirmed analytically and are therefore based on nominal treatment levels. However, measured residues of afidopyropen immediately (<4 h) following application in *Phacelia* flowers and leaves were 3.34 ± 0.27 and 1.66 ± 0.18 mg a.i./kg, respectively; afidopyropen residues in flowers were significantly ($p<0.05$) higher than residues in leaves. Measured residues of the transformation product M4401007 in flowers and leaves were 2.75 ± 0.16 and 3.30 ± 0.28 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar samples following applications (1 DAT) were 0.06 mg a.i./kg and <LOQ (0.006 mg a.i./kg), respectively; M440I007 residues in pollen and nectar specimens were 0.08 mg/kg and <LOQ, respectively.

Afidopyropen treatments resulted in significantly ($p < 0.05$) different (i.e., 38% higher) mean adult worker bee mortality (15.69 dead adult worker bees/colony/day) relative to control treatments (11.40 dead adult worker bees /colony/day) after applications were made (*i.e.,* including both exposure and monitoring phases). Mean mortality of pupae in afidopyropen-treated colonies was roughly similar to that in control colonies throughout the study. Mean foraging activity in afidopyropen-treated colonies during the exposure phase of the study (16.78 bees/m^2/colony/d) was significantly ($p < 0.05$) different (*i.e.*, 12% lower) than mean foraging activity in control colonies (19.14 bees/m^2/colony/d). There were no significant differences in colony strength (mean no. of adult bees or pupae/colony/d) or condition (mean no. of cells as brood [eggs and larvae] or food [honey and pollen]) in afidopyropen-treated colonies relative to control colonies. Afidopyropen treatments also resulted in sublethal behavioral effects after application on the day of treatment (0aa DAT), wherein roughly 50 bees/tunnel displayed loss of coordination and lethargic behavior in the dead zone dead bee trap. One to four days after treatment (DAT) the study author reported that "few" bees (in each tunnel) were observed to fall from flowers while foraging.

Results Synopsis:
The study is generally consistent with OECD Guidance Document No. 75, although there are some potentially important study deviations and deficiencies. As treatment levels were not analytically

[1] Note that all means in this summary are followed by ± one standard error (SE).

verified in the study, and due to possible effects of weather prior to and immediately following applications, there is uncertainty regarding actual afidopyropen exposure levels.

Honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) exhibited significant ($p<0.05$) increases in adult worker bee mortality and decreases in foraging activity, resulting in a no-observed adverse effect level (NOAEL) of <50 g a.i./ha under the conditions tested. While there was increased adult worker bee mortality following afidopyropen applications, and decreased foraging activity during the test item exposure phase of the study, at the conclusion of the study there were no significant differences in juvenile survival, or colony strength and condition in afidopyropen-treated colonies relative to control colonies. Therefore, the increased mortality in adult bees and decrease foraging activity following application of afidopyropen appear to be transient effects.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

I. DATA SOURCE

USEPA MRID No.:	49689235
PMRA UKID No.:	2627509
Study Title:	Semi-field brood study to evaluate potential effects of BAS 440 00 I on the development of honeybee colonies (*Apis mellifera*)
Study Author(s):	Staffel J.
Testing Laboratory:	RIFCON GmbH, Goldbeckstraße 13, 69493 Hirschberg, Germany
Laboratory Report No.:	737518
Sponsor Study No.:	BASF Reg. Doc. #: 2015/1005007
Study Completion Date:	14 December 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes

II. MATERIALS AND METHODS

Test Guideline: OECD Guidance Doc. No. 75 (2007)

Deviations from Guideline:

- The quantities of material applied in both the test item (afidopyropen) and the reference item (dimethoate) treatments was not verified analytically.
- The acclimation period for honey bee colonies in this study (6 days) is longer than what is recommended (2-3 days) in OECD Guidance Document No. 75; though not explicitly stated by the study author, weather data indicate that it rained several days before applications were made, which could explain the extended acclimation period (see Reviewer's Comments for additional discussion).
- The study methodology for the collection of pollen samples and nectar in honey bee stomachs for the analysis of afidopyropen residues did not provide for the collection of replicate samples within the single 'residue' tunnel (tunnels used to monitor residues for afidopyropen and control tunnels were separate from those used to assess effects); instead only a single pooled sample was taken from the control and the test item-treated tunnel, respectively.
- The post-application pollen trap sample for the afidopyropen residue tunnel collected 1 DAT was supplemented with pollen collected directly from forager bees, and also from pollen

collected inside the tunnel's hive 3 DAT; therefore, this sample really represents a combined sample for 1-3 DATs.

- For the following time points, the maximum daily temperature exceeded the recommended maximum daily temperature in the OECD guidance document (30.0 °C): 0, 1, 4-10, 12, 22, 27, and 39. In particular, 7 DAT (the day that bees were moved to the monitoring location) the maximum temperature reached 39.6 °C; the mean daily temperature 7 and 10 DATs was just shy of 30.0 °C, suggesting elevated overall temperatures throughout the day.

GLP Compliance: Yes; signed GLP certificate was included and reported no guideline deviations. Laboratory certified by the LUBW Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg, Karlsruhe.

A. MATERIALS

Test Material: BAS 440 00 I (VERSYS™)

Test Material Identity Batch No. FD-130925-0022; a yellow, liquid formulation comprising afidopyropen (BAS 440 I): 100 g/L (nominal), 98.2 g/L (9.8% measured).

Details on Preparation and Application of Test Materials:
All substances were applied in 400 L/ha water using a calibrated, portable boom sprayer (250 cm wide, 50 cm between nozzles).

Analytical Monitoring: None reported.

Details on Analytical Monitoring: *N/A*

Reference material: Perfekthion® (formulated dimethoate: 400 g/L (nominal)

Reference Material Identity Batch 0001100403; blue liquid

Vehicle: None

Test Organism (Species): *Apis mellifera* L. (honeybee)

Animal Group: Arthropoda/Insecta/Hymenoptera/Apidae

Details on Test Organisms: Healthy honeybee colonies, containing ten combs consisting of three to five brood combs including all brood stages and sufficient food supply, were used for the study. At the first brood assessment, *i.e.*, brood fixation day zero (BFD 0) two days prior to treatment (-2 DAT), colonies contained 18,000 to 28,400 brood cells with all stages present; 11,800 to 21,200 food cells; and approximately 5,005 to 12,220 adult bees. Bees in the colonies were free of clear visual signs of disease or pests, and no unusual occurrences were reported in colonies prior to treatments. Sister queens from 2014 were used to produce colonies which were as uniform as possible (source: RIFCON GmbH, Hirschberg, Germany).

B. STUDY DESIGN AND METHODS

Study Type: Semi-field (tunnel) study

Test Duration Type: Long-term (41 d) toxicity test

Limit Test: None reported

Total Exposure Duration: 7 d

Post-Exposure Observation Phase: 34 d

Remarks: Bee mortality was assessed daily beginning three days before (-5 DAT) and ending 33 days after treatment (33 DAT). Mortality in the tunnels was evaluated using linen sheets (area approximately 18 m^2) laid at ground level inside the front, middle and back of the tunnels, as well with dead zone dead bee traps at each hive entrance; mortality at the monitoring site was evaluated using only dead zone dead bee traps. Foraging activity of the bees, and overall behavior, were assessed 5 days before to 7 days after application (-5 to 7 DAT). Condition of the colonies (food stores, brood status and colony strength) were assessed -1, 4, 11, 22, 32, and 41 DAT. Colony assessments were conducted according to the Liebefeld method[2,3,4]; for this purpose, both sides of all combs in each hive were visually divided into 1 dm^2 areas. One (100 cm^2) square covered densely with honeybees was assumed to represent ~130 worker bees or ~400 worker bee cells, respectively, and one square of male brood was assumed to contain ~230 cells[2,3,4]. The absolute number of honeybees and cells filled with brood or food was calculated by multiplying the number of estimated squares by 130 (for honeybees), by 400 (for worker bee cells containing brood or food), or by 230 (male brood cells). Afidopyropen residues in flowers and leaves were assessed using samples collected from all afidopyropen and control tunnels before and after (<4 h) treatments; residues in pollen (-1 and 1 DAT) and in nectar from the honey stomach of forager bees (-1 and 1 DAT) were assessed using samples collected from two additional 'residue-only' tunnels.

Test Environmental Conditions:

Ambient environmental conditions inside the tunnels (weather data for -3 to 7 DAT within tunnel #2 of the negative control treatment group, data for 8 to 41 DAT acquired at the monitoring site) and reported here as daily means: 13.3-16.7 °C and 76.3-89.4% relative humidity (RH) before application; 20.6-26.3 °C and 36-53% RH during application; 19.2-29.7 °C and 53.1-79.0% RH during the 7-d exposure phase in the tunnels; 15.2-33.1°C and 36.5-81.7% RH during the 34-d monitoring phase. Rainfall (>1.0 mm) was reported during the study on -4, -3, -2, -1, 2, 3, 13, 14, 19, 23, 24, 25, 28, 30, 32, 34, 35, and 41 DAT, and consisted of 1.0, 7.0, 11.0, 3.0, 10.0, 5.0, 4.0, 1.0, 1.0, 11.0, 1.5, 13.0, 1.0, 2.0, 5.5, 3.5, 1.0, 1.0, and 3.0 mm, respectively.

Photoperiod and Lighting: Natural

Nominal and Measured Concentrations:

Negative control: tap water (400 L/ha)
Afidopyropen: 0.5 L/ha (50 g a.i./ha (nominal))
Dimethoate: 1.2 L/ha (480 g a.i./ha (nominal))

[2] Aumeier P. 2008. 10, 20 oder 35 Tausend im Volk? ADIZ/db/IF 4/2008.
[3] Imdorf A and Gerig L. 1999. Lehrgang zur Erfassung der Volksstrake. Schweizerisches Zentrum fur Bienenforschung.
[4] Imdorf A, Buehlmann G, Gerig L, Kilchenmann V, and Wille H. 1987. Uberprufung der Schatzmethode zur Ermittlung der Brutflache und der Anzahl Arbeiterinnen in freifliegenden Bienenvolkem. Apidologie 18: 137:146.

Test Plots: The test site was located in 68526 Ladenburg, Baden-Württemberg, Germany. Separate tunnels were used for the three treatment groups (afidopyropen, dimethoate, water). Tunnels (18 m length x 6 m width x 2.9 m height [108 m² floor space]) were set up within a field of *P. tanacetifolia*.

Test Design: Tunnel test under semi-field conditions, study was carried out using four tunnels (*i.e.*, replicates) for each treatment group, with one bee hive per 108 m² tunnel. Tunnels were set up on a field of *P. tanacetifolia*, and healthy bee colonies were introduced on 18 June 2015, shortly before full flowering of the crop, and six days before application (DAT -6). The application was carried out during bee flight at full flowering of the crop. Bees were exposed to the water-, afidopyropen- or dimethoate-treated phacelia in the tunnels for seven days. Seven days after applications, colonies were removed from the tunnels and relocated to a monitoring site approximately 5.75 km west. The monitoring site (near Hirschberg, Baden-Württemberg, Germany) was located in a forested area with no bee-attractive crops.

Assessments of the persistence of afidopyropen residues in *P. tanacetifolia* flowers, leaves, pollen (pollen traps and directly from bees), and in nectar from the honey stomach of foraging bees, were carried out treatment tunnels and in separate residue-monitoring tunnels simultaneous to tests for effects on honey bee brood development. Residues in flowers and leaves were measured in both the four treated tunnels (C1-4 and T1-4) in addition to the 'residue only' tunnel for afidopyropen, while pollen and nectar samples were collected only in the single 'residue only' tunnel. Residues in whole flowers and leaves were assessed using samples collected from test item and control tunnels before applications (sampling split between -6 and -1 DAT), and after applications (<4 h). A composite sample (≥5 g each) of flower blossoms and leaf tissues were randomly collected from each of the test item and control tunnels (4 x), and stored at ≤-18 °C within 6 h of collection. Pollen samples (≥1 g) were collected before (-1 DAT) and after (1-3 DAT) applications in the 'residue only' tunnel, using a pollen trap attached to the tunnel's hive to collect pollen from honeybee pollen loads; as not enough pollen could be collected this way additional samples were collected from collected forager bees and from inside the tunnel's hive. Foraging bees (approx. 300 bees/tunnel) for honey stomach analysis were collected -1 and 1 DAT inside the residue tunnels using a modified hand-held vacuum. Collected bees were frozen until dissection, when they were defrosted so that stomachs could be removed; collected honey stomachs were then stored at ≤-18 °C. All collected samples were shipped on dry ice to SGS Institut Fresenius GmbH (Taunusstein, Germany) for residue analysis.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration:	7 d
Endpoint(s):	No effect level
Effect Concentration:	≥ 0.5 L/ha
Basis for Concentration:	Nominal
Effect Concentration Type:	Test material
Basis for Effect:	Effects observed for the following endpoints: survival of adult bees and pupae, foraging activity, behavior, colony development, colony strength, bee brood.

Applicant-Provided Results:

Application Conditions & Deviations: Applications were made using two identically-equipped hand-held boom sprayers (one for the control and reference item, the other for the test item) between 11:23 and 12:54 hrs. Bee foraging activity prior to applications was reported to be 14.3-30.0 bees/m^2 in study tunnels. Wind speed outside tunnels was 0.0-0.5 m/s, temperature was 20.6-26.3 °C, and relative humidity was 36-53%. The amount of applied product (based on application volumes) deviated from the target application amount by –0.4 to 0.2% for test item applications, and -0.7 to 0.8% for reference item applications.

Sublethal Behavioral Effects: According to the study authors, there were no reported observations of sublethal behavioral effects in control tunnels at any time during the study (see **Appendix II** for summary table provided by study author). In tunnels receiving afidopyropen treatments, after application on the day of treatment (0aa DAT), roughly 50 bees/tunnel displayed loss of coordination and lethargic behavior in the dead zone dead bee trap. One to four days after treatment (DAT) the study author reported that "few" bees (in each tunnel) were observed to fall from flowers while foraging. In a single control tunnel (#4), roughly 200 bees where reported to cluster in front of the hive and dead zone dead bee trap 7 DAT. In tunnels receiving dimethoate treatments, the following behavioral effects were reported by the study authors: cramping, coordination problems, symptoms of intoxication (*i.e.*, problems landing, issues with nectar uptake, dropping to ground during flight), and clustering just outside the hive.

Adult & Juvenile Mortality: According to the study author, adult bee mortality in dimethoate-treated colonies was significantly ($p < 0.05$) different (*i.e.*, higher) than controls during all phases of the study; there were apparently no differences in adult bee mortality in afidopyropen-treated colonies relative to the control (see **Table 1**). The study author did not statistically analyze data on mortality of pupae due to low overall mortality (<0.7 dead pupae/colony/day) in all treatments groups.

Table 1. Study author-reported effects on bee (*Apis mellifera*) mortality, foraging activity, and bee brood development under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and dimethoate (reference)-treated colonies (means ± standard deviation are reported).

	Control	Afidopyropen	Dimethoate
Mean mortality of adult worker bees (n dead bees/colony/day)			
Pre-application phase [1]	55.3 ± 16.1	77.0 ± 43.3	**88.7 ± 49.3 †**
Exposure phase in the tunnels [1]	29.2 ± 19.3	29.4 ± 11.5	**339.0 ± 441.0 †**

Monitoring phase outside the tunnels [2]	7.4 ± 7.4	7.0 ± 7.6	**26.4 ± 31.9 †**
Overall after application	11.7 ± 13.9	11.4 ± 12.2	**87.4 ± 230.7 †**
Mean mortality of pupae (n dead pupae/colony/day) [3]			
Pre-application phase [1]	0.3 ± 0.9	0.3 ± 0.6	0.7 ± 1.0
Exposure phase in the tunnels [1]	0.4 ± 1.1	0.2 ± 0.4	0.3 ± 0.9
Monitoring phase outside the tunnels [2]	0.0 ± 0.1	0.1 ± 0.2	0.3 ± 1.0
Overall after application	0.1 ± 0.5	0.1 ± 0.3	0.3 ± 1.0
Mean foraging activity/m²/colony/day [n]			
Pre-application phase	12.3 ± 7.0	11.6 ± 7.7	13.9 ± 7.6
Exposure phase in the tunnels	19.2 ± 7.7	**16.8 ± 6.9 †**	3.0 ± 7.9

[1)] Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
[2)] Mean number of dead honeybees per day and colony found in dead bee traps, only.
[3)] Data on mean mortality of pupae was not statistically analyzed by the study author.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's t test
† = statistically significant differences ($p < 0.05$) compared to the control, pairwise Mann-Whitney test
DAT = days after treatment

Colony Strength: The study author did not appear to statistically analyze colony strength data, but nevertheless stated that while there was no indication of adverse effects from afidopyropen treatments, dimethoate treatments appeared to show adverse effects with lower colony strength (relative to the control) during the monitoring phase of the study. The mean number of bees per colony (across all treatment groups) prior to applications (-1 DAT) was 7,908 bees (**Table 2**).

Table 2. Summary of colony strength (mean number of worker bees) in control, afidopyropen (test item) and dimethoate (reference) colonies at specified days after application (DAA). Table reproduced from BASF Study ID 2015/1005007.

Date [dd.mm. yyyy]	DAA	Control group			Test item group			Reference item group		
		absolute [n] [1)]		Relative	absolute [n] [1)]		Relative	absolute [n] [1)]		Relative
		mean	± SD	develop-ment [2)]	mean	± SD	develop-ment [2)]	mean	± SD	develop-ment [2)]
24.06.2015	-1	7,215	1,268	-	7,329	2,056	-	9,181	2,051	-
29.06.2015	4	8,011	267	+11 %	7,833	1,093	+7 %	4,973	1,201	-46 %
06.07.2015	11	11,716	1,346	+62 %	12,968	4,215	+77 %	7,881	1,399	-14 %
17.07.2015	22	11,229	1,179	+56 %	11,083	2,852	+51 %	6,906	2,264	-25 %
27.07.2015	32	14,446	1,309	+100 %	13,553	1,586	+85 %	10,010	3,898	+9 %
05.08.2015	41	13,764	1,281	+91 %	13,683	1,515	+87 %	9,896	3,458	+8 %

DAA = days after application; [1)] absolute mean strength of the colonies ± standard deviation; [2)] relative development of the mean strength of the colonies (strength of the colonies at the first assessment was set as basis)

Foraging Activity: According to the study authors, mean foraging behavior in the afidopyropen-treated colonies was significantly ($p < 0.05$) different (*i.e.*, 12% lower) than controls during the exposure phase of the study (see **Table 1**); otherwise, there were no significant difference in foraging activity from afidopyropen or dimethoate treatments relative to the negative control.

Colony Condition: According to the study authors, the evaluation of brood at -1 DAT indicated healthy colonies with queens and all brood stages present, and a sufficient supply of nectar and pollen (see **Tables 3** and **4**). The study author did not appear to statistically analyze colony condition data, but nevertheless stated that while there was no indication of adverse effects from afidopyropen treatments, dimethoate treatments appeared to show adverse effects with fewer brood cells (relative to the control) during the end of the exposure phase and through the midway point of the monitoring phase of the study. The study author did not report any adverse effects from either afidopyropen or dimethoate treatments with respect to average quantity of food cells. Food supplies were reportedly supplemented 33 DAT with 500 g Nektapoll (a commercially available protein/fructose [patty] supplement) and 2500 g Apifonda (sucrose paste).

Table 3. Summary of total number of brood (eggs, larvae and pupae) in control, afidopyropen (test item) and dimethoate (reference) colonies at specified days after application (DAA). Table reproduced from BASF Study ID 2015/1005007.

Date [dd.mm. yyyy]	DAA	Control group			Test item group			Reference item group		
		Absolute [n] [1]		Relative develop-ment [2]	Absolute [n] [1]		Relative develop-ment [2]	Absolute [n] [1]		Relative develop-ment [2]
		Mean	± SD		Mean	± SD		Mean	± SD	
24.06.2015	-1	23,600	3,767	-	22,450	3,678	-	23,100	3,519	-
29.06.2015	4	22,600	1,911	- 4 %	20,000	1,095	-11 %	12,700	4,703	-45 %
06.07.2015	11	19,500	1,793	-17 %	16,100	1,501	-28 %	7,750	5,529	-66 %
17.07.2015	22	21,600	952	-8 %	21,550	1,012	-4 %	14,900	5,176	-35 %
27.07.2015	32	23,700	2,295	±0 %	22,050	1,330	-2 %	17,700	5,754	-23 %
05.08.2015	41	19,100	2,543	-19 %	19,400	952	-14 %	17,750	4,110	-23 %

DAA = days after application; [1] absolute mean strength of the colonies ± standard deviation; [2] relative development of the mean strength of the colonies (strength of the colonies at the first assessment was set as basis)

Table 4. Summary of total number of food (honey and pollen) cells in control, afidopyropen (test item) and dimethoate (reference) colonies at specified days after application (DAA). Table reproduced from BASF Study ID 2015/1005007.

Date [dd.mm. yyyy]	DAA	Control group			Test item group			Reference item group		
		Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]	Absolute [n] [1)]		Relative develop-ment [2)]
		Mean	± SD		Mean	± SD		Mean	± SD	
24.06.2015	-1	14,900	4,266	-	14,150	2,537	-	16,000	2,866	-
29.06.2015	4	13,200	3,472	-11 %	12,950	2,620	-8 %	14,250	2,357	-12 %
06.07.2015	11	15,500	2,783	+4 %	15,700	3,139	+11 %	13,600	2,321	-15 %
17.07.2015	22	12,250	1,792	-18 %	12,250	2,402	-13 %	9,700	2,017	-39 %
27.07.2015	32	7,350	1,136	-51 %	7,600	1,883	-46 %	6,250	2,408	-61 %
05.08.2015	41	11,000	2,790	-26 %	11,750	4,145	-17 %	9,800	2,546	-39 %

DAA = days after application; [1)] absolute mean food stores ± standard deviation; [2)] relative development of food stores (food stores at the first assessment was set as basis)

Residues: The study author reported that no residues of either BAS 440 I (afidopyropen) or ittransformation product M440I007 were found in flower, leaf, nectar or pollen specimens collected at random locations in tunnels before applications were made. No residues of either compound were reportedly found in specimens collected in negative control treatment tunnels following applications. Immediately (<4 h) following applications afidopyropen residues in *Phacelia* flowers and leaves were 2.84-4.09 and 1.24-2.21 mg a.i./kg, respectively; M440I007 residues in flowers and leaves were 2.25-3.17 and 2.45-3.82 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar specimens were 0.06 mg a.i./kg and <0.003 mg a.i./kg (Limit of Quantification; LOQ), respectively; M440I007 residues in pollen and nectar specimens were 0.08 mg a.i./kg and <0.003 mg a.i./kg (LOQ), respectively.

Weather Data: Weather data reported by the study author is summarized in **Figure 1**, and includes total daily precipitation (mm), daily mean temperature (°C), and daily mean humidity (% RH). The study author noted that prior to applications substantial rainfall (7, 11 and 3 mm, respectively) occurred between three days and one day before applications (-3 to -1 DAT). Minimum daily temperatures during the pre-application phase were 8.4 (-2 DAT) – 12.4 (-3 DAT) °C, and maximum daily temperatureswere 16.4 (-3 DAT) – 25.1 (-1 DAT) °C. During the exposure phase of the study, substantial rainfall (10 and 5 mm, respectively), occurred 2 and 3 DATs. Minimum daily temperatures during the exposure phase were 9.2 (0 DAT) – 18.1 (7 DAT) °C, and maximum daily temperatures were 29.6 (3 DAT) – 39.6 (7 DAT) °C. During the monitoring phase of the study rainfall (4.0, 11.0, 1.5, 13.0, 2.0, 5.5, 3.5 and 3.0 mm) occurred 13, 23, 24, 25, 28, 30, 32 and 41 DATs. Minimum daily temperatures were 10.0 (36 DAT) – 25.7 (9 DAT) °C, and maximum daily temperatures were 18.2 (34 DAT) – 35.1 (10 DAT) °C.

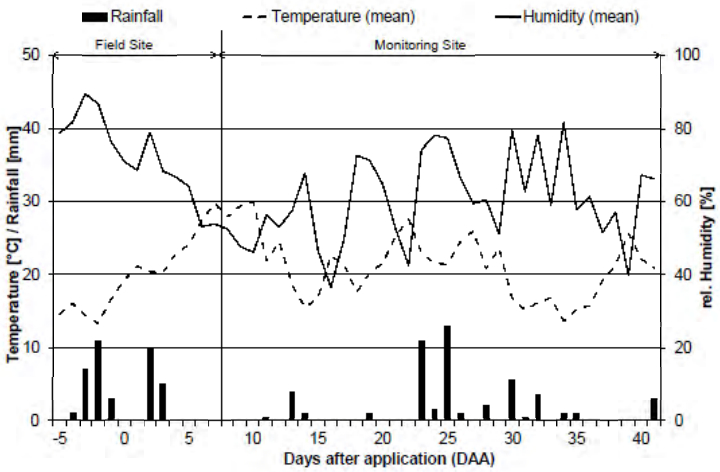

Figure 1. Weather data (rainfall, temperature and humidity) reported by the study author.

Overall, the study author concluded that BAS 440 00 I did not adversely affect honeybee colonies in this study.

Applicant-Reported Statistics and Error Estimates
The applicant reported means and standard deviations for all endpoints, included calculated brood indices. R (ver. 3.0.3) was used for all of the study author's statistical analyses.

The applicant statistically analyzed the following endpoints: mortality, and overall foraging activity; both datasets were initially tested for parametric test assumptions (*i.e.*, using Shapiro-Wilk's and Bartlett's tests). Depending on the results of assumptions tests, mortality data were analyzed with ANOVA and Dunnett's multiple means test, or Kruskal-Wallis and Mann-Whitney U tests; foraging data were analyzed with Student's t, Welch, or Mann-Whitney U tests. All pre-application comparisons were made using two-sided tests, and all post-application comparisons were made using one-sided tests (*i.e.*, "greater" for mortality and "smaller" for foraging activity. Data on foraging activity from -2 and -3 DAT were excluded from statistical analyses due to unfavorable weather conditions.

IV. OVERALL REMARKS, ATTACHMENTS

Microsoft Excel data tables were submitted with an OECD-formatted summary by the registrant. The applicant did not include raw data on measured residues in the provided Excel tables, and so these data were manually extracted from the study report by the reviewer.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

The reviewer verified all of the applicant's calculations and carried out statistical analyses per relevant EFED guidance for all data to confirm the applicant's results and conclusions.

Adult & Juvenile Mortality: Mean adult honey bee mortality was significantly ($p < 0.05$) different (*i.e.*, 38% higher) overall following applications of afidopyropen compared to control tunnels (afidopyropen:

15.69 dead bees/colony/d; control: 11.40 dead bees/colony/d). Mean adult honey bee mortality in dimethoate-treated tunnels was significantly ($p < 0.05$) different (*i.e.*, 12x higher) compared to negative control tunnels during the exposure (dimethoate: 301.31 dead bees/colony/d; control: 25.89 dead bees/colony/d) and 3.5x higher during monitoring periods (dimethoate: 26.42 dead bees/colony/d; control: 7.45 dead bees/colony/d) of the study (and therefore also overall post-applications). Otherwise, there were no significant differences in adult bee mortality between afidopyropen- and dimethoate-treated groups and the negative control during the study (**Table 5**).

During the monitoring period of the study, mean mortality of pupae was significantly ($p < 0.05$) different (*i.e.*, 16x higher) in dimethoate-treated tunnels compared to control tunnels (dimethoate: 0.31 dead pupae/colony/d; control: 0.02 dead pupae/colony/d) (**Table 5**).

Table 5. Reviewer-calculated effects on honey bee (*Apis mellifera*) mortality (juvenile & adult worker) and foraging activity under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for negative control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and dimethoate (reference)-treated colonies (means ± standard error are reported).

	Control	Afidopyropen	Dimethoate
Mean mortality of adult worker bees (n dead bees/colony/day)			
Pre-application phase [1]	55.25 ± 3.28	77.00 ± 8.84	88.71 ± 40.06
Exposure phase in the tunnels [1]	25.89 ± 3.30	26.08 ± 1.92	**301.31 ± 42.71 †**
Monitoring phase outside the tunnels [2]	7.45 ± 0.65	5.31 ± 0.76	**26.42 ± 2.78 †**
Overall after application [3]	11.40 ± 1.04	**15.69 ± 1.60 †**	**85.32 ± 1.04 †**
Mean mortality of pupae (n dead pupae/colony/day)			
Pre-application phase [1]	0.29 ± 0.18	0.33 ± 0.13	0.67 ± 0.21
Exposure phase in the tunnels [1]	0.33 ± 0.18	0.14 ± 0.06	0.28 ± 0.15
Monitoring phase outside the tunnels [2]	0.02 ± 0.01	0.03 ± 0.03	**0.31 ± 0.08 †**
Overall after application	0.09 ± 0.04	0.08 ± 0.03	**0.30 ± 0.07 †**
Mean foraging activity (bees/m^2/colony/day [n])			
Pre-application phase[4]	8.27 ± 1.61	7.70 ± 1.68	9.47 ± 1.69
Exposure phase in the tunnels	19.14 ± 0.89	**16.78 ± 0.78 †**	**3.21 ± 1.00†**

[1)] Sum of dead individuals found in dead bee traps and on linen sheets in the tunnels.
[2)] Mean number of dead honeybees per day and colony found in dead bee traps.
[3)] 'Overall after application' value for the reference item treatment group only includes data from the monitoring period of the study.
[4)] The study author excluded data collected on -3 and -2 DATs from this calculation due to heavy rainfall on these two dates.
* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test
† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon Rank Sum test

Foraging Activity: Mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 24% lower) in afidopyropen (16.78 bees/m^2/colony/d) tunnels and 7x-lower in dimethoate (3.21 bees/m^2/colony/d) tunnels compared to control tunnels (22.10 bees/m^2/colony/d) during the exposure period of the study; otherwise, there were no significant differences in foraging activity between treatment groups and the control during the study (**Table 5**).

Colony Strength: At 4 and 22 DAT the mean number of worker bees in dimethoate tunnels was significantly ($p<0.05$) different (*i.e.*, higher) than the mean number of worker bees in the control tunnels (**Table 6**). The mean number of adult worker bees in afidopyropen-treated tunnels was similar to that in control tunnels throughout the study.

The mean number of pupae in dimethoate-treated tunnels was significantly ($p<0.05$) different (*i.e.*, lower) than the mean number of worker bees in the control tunnels at 4, 11 and 32 DATs; otherwise, there were no significant differences in the mean number of pupae between the afidopyropen and dimethoate treatment groups and the negative control during the study (**Table 6**).

Colony Condition: There were no statistically significant differences in the overall quantity of brood or food cells (*i.e.*, honey and pollen) in afidopyropen or dimethoate-treated colonies relative to control colonies at any time during the study (**Table 6**).

Table 6. Reviewer-calculated effects on honey bee (*Apis mellifera*) colony strength and condition under semi-field conditions (tunnel test) at pre-application, in-tunnel exposure phase, and post-exposure monitoring phase for control, formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated, and dimethoate (reference)-treated colonies (means ± standard error are reported).

	Days After Treatment (DAT)					
	-1	**4**	**11**	**22**	**32**	**41**
Colony Strength – Adults (n adult bees/colony/d)						
Control	6858 ± 743	7719 ± 190	11213 ± 557	10416 ± 561	13829 ± 518	13033 ± 449
Afidopyropen	7215 ± 1091	7589 ± 513	12220 ± 1872	9929 ± 1174	12935 ± 606	12903 ± 642
Dimethoate	8808 ± 890	**4745 ± 476 ***	7751 ± 694	**6549 ± 1027 ***	9458 ± 1832	10221 ± 1644
Colony Strength – Juveniles (n juveniles/colony/d)						
Control	15200 ± 1192	14700 ± 1201	9750 ± 780	11550 ± 830	13850 ± 499	9600 ± 1175
Afidopyropen	14300 ± 1103	11650 ± 950	7000 ± 990	11400 ± 787	12300 ± 755	7650 ± 544
Dimethoate	14500 ± 1310	**9800 ± 455 ***	**2100 ± 656 ***	6500 ± 2391	**9650 ± 1473 ***	8850 ± 838
Colony Condition – Brood (n cells/colony/d as brood)						
Control	2800 ± 771	2633 ± 255	3250 ± 705	3300 ± 812	3283 ± 743	3167 ± 775
Afidopyropen	2717 ± 673	2783 ± 677	3033 ± 675	3383 ± 903	3250 ± 731	3917 ± 925
Dimethoate	2876 ± 673	967 ± 480	1883 ± 683	2800 ± 648	2683 ± 677	2967 ± 751
Colony Condition – Food (n cells/colony/d as food)						
Control	7450 ± 2329	6600 ± 1704	7750 ± 1671	6125 ± 1442	3675 ± 949	5500 ± 1059
Afidopyropen	7075 ± 1983	6475 ± 1614	7850 ± 1532	6125 ± 1329	3800 ± 1106	5875 ± 1567
Dimethoate	8000 ± 2635	7125 ± 2184	6800 ± 2063	4850 ± 1249	3125 ± 1021	4900 ± 1136

* = statistically significant differences ($p < 0.05$) compared to the control, Dunnett's test
† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon Rank Sum test

Residues: Note that for analysis of afidopyropen residues in flowers and leaves, a single sample was collected from each of the 4 negative control tunnels, and for each of the 4 afidopyropen tunnels in addition to a separate residue sampling only with an afidopyropen tunnel (*i.e.*, this tunnel was not used for biological effects data), allowing for statistical analysis of these treatment means; samples for analysis of residues in pollen and nectar were collected from the single residue sampling only test item tunnel, so no analyses could be carried out on reported residue results for nectar and pollen residues.

Residues of parent afidopyropen (BAS 440 I) and its metabolite (M440I007) were below the analytical level of detection (LOD = 0.003 mg a.i./kg) in leaves and flowers collected both before and after

applications in all negative control treatment tunnels. Similarly, residues of both compounds were below the LOD in afidopyropen-treated tunnels prior to applications. Immediately (<4 h) following applications afidopyropen residues in *Phacelia* flowers and leaves were 3.34 ± 0.27 and 1.66 ± 0.18 mg a.i./kg, respectively; afidopyropen residues in flowers were significantly ($p<0.05$) higher than residues in leaves. M440I007 residues in flowers and leaves were 2.75 ± 0.16 and 3.30 ± 0.28 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar samples following applications (1 DAT) were 0.06 mg a.i./kg and <LOQ (0.006 mg a.i./kg), respectively; the photo-dimer M440I007 residues in pollen and nectar specimens were 0.08 mg/kg and <LOQ, respectively.

Reviewer's Statistical Verification:
Statistical analyses confirmed using R (ver. 3.2.5)[5] statistical software, and the multcomp[6] analysis package. The reviewer relied on the Shapiro-Wilk's test and Bartlett's test to evaluate whether data were normally distributed or homoscedastic, respectively. ANOVA and Dunnett's Multiple Means test was used to test for statistical differences amongst means for data that met assumptions for parametric tests (*i.e.,* data were approximately normally distributed and had homogenous variances), and Kruskal-Wallis and Wilcoxon Rank Sum test was used for non-parametric means comparisons. One-sided tests were used for all hypothesis-based testing; $\alpha = 0.05$ for all mean comparison tests, and $\alpha = 0.01$ for all assumptions testing.

See **Appendix I** for summary statistics and diagnostic tests (*i.e.,* goodness-of-fit and equivalent variances tests) for all data described in this data evaluation report.

Based on statistically significant effects on adult worker honeybee mortality and foraging activity in afidopyropen-treated colonies, the no-observed adverse effect level (NOAEL) across the various measurement endpoints for adult honey bees and developing brood is <50 g a.i./ha under the conditions tested.

Reviewer's Comments:
The reviewer's overall results and conclusions for adult mortality and foraging activity agreed with those of the study author, in spite of some differences regarding the exclusion of data points in the later data set. The study author did not statistically analyze any of the other endpoints for which data were collected, so comparisons between the reviewer's and study author's conclusions for these endpoints is not possible.

Data provided in the study report indicate that the average time to make applications to each tunnel was 2 minutes per tunnel (range was 1-4 minutes for control treatments, 1-2 minutes for afidopyropen treatments, and 1 minute for dimethoate treatments). Given the described application protocols in the study report it's difficult to understand how applications could have been made to each of the tunnels in such a short timeframe.

The study author excluded foraging behavior data collected on -2 and -3 DAT due to unfavorable weather conditions that apparently substantially reduced overall foraging activity of honeybees across

[5] R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
[6] Hothorn T, F Bretz and P Westfall. 2008. Simultaneous inference in general parametric models. Biometric Journal 50: 346-363.

all treatment groups. The reviewer included these data; while the reviewer agrees that unfavorable weather conditions may have adversely impacted foraging activity, the collected data represent responses of honeybee communities to environmental variability to afidopyropen treatments in the context of real world conditions, and therefore the dataset was evaluated in its entirety.

For a seven-day period (4-10 DAT) spanning the end of the in-tunnel exposure phase and the beginning of the remote monitoring phase, the maximum daily recorded temperature was 32.1-39.6 °C. OECD Guidance Document No. 75 notes that daytime temperatures exceeding 30 °C may stop nectar secretion. Additionally, rainfall exceeding 10 mm was reported several times during the study (-2, 2, 23, and 25 DAT)), and rainfall –3, 3, 13, 30, 32 and 41 DAT exceeded 3 mm. Excessive precipitation was implicated by the study author in "severely" reduced honey bee foraging activity -3 and -2 DAT (leading to the study author excluding data from these days from their analyses).

Study results indicate that the reference item (dimethoate) resulted in the following significant ($p < 0.05$) adverse effects relative to negative control colonies: increased adult worker bee mortality during the exposure and monitoring phases of the study; increased mortality of pupae during the monitoring phase of the study; reduced foraging activity during the exposure phase of the study; lower mean number of adult worker bees at 4 and 22 DATs; and, lower mean number of pupae at 4, 11 and 32 DATs. These responses due to dimethoate treatment suggest that honeybee colonies in this study were exposed to test materials and that the test system was able to detect treatment effects associated with the reference toxicant.

Reviewer's Conclusions:
The semi-field (tunnel) bee brood study was initiated in June 2015 with the formulated end-use product BAS 440 00 I (VERSYS™, 9.8% afidopyropen). Bee colonies in the negative control, reference item (dimethoate: 480 g a.i./ha nominal), and 50 g a.i./ha BAS 440 00 I treatments were assessed at multiple time points; treatment rates were not confirmed analytically; however, residues in various matrices (leaves, flowers, pollen and nectar) were measured. The exposure phase was seven days (0 – 7 DAT), and the post-exposure monitoring phase 34 days (8 – 41 DAT).

In summary, afidopyropen treatments resulted in significantly ($p < 0.05$) different (*i.e.*, 38% higher) mean adult worker bee mortality (15.69 dead adult worker bees/colony/day) relative to control treatments (11.40 dead adult worker bees /colony/day) after applications were made (*i.e.* including both exposure and monitoring phases). Mean mortality of pupae in afidopyropen-treated colonies was roughly similar to that in control colonies throughout the study. Mean foraging activity in afidopyropen-treated colonies during the exposure phase of the study (16.78 bees/m^2/colony/d) was significantly ($p < 0.05$) different (*i.e.*, 12% lower) than mean foraging activity in control colonies (19.14 bees/m^2/colony/d). There were no significant differences in colony strength (mean no. of adult bees or pupae/colony/d) or condition (mean no. of cells as brood [eggs and larvae] or food [honey and pollen]) in afidopyropen-treated colonies relative to control colonies. Finally, afidopyropen treatments resulted in sublethal behavioral effects after application on the day of treatment (0aa DAT), wherein roughly 50 bees/tunnel displayed loss of coordination and lethargic behavior in the dead zone dead bee trap. One to four days after treatment (DAT) the study author reported that "few" bees (in each tunnel) were observed to fall from flowers while foraging.

There were inclement weather conditions during the pre-application period (*i.e.*, rainfall -3 to -1 DAT totaled roughly 21 mm), and 4-10 DAT (spanning the exposure and monitoring phases of the study) with average daily temperatures of 23-30 °C. On the seventh day after treatment the maximum temperature was 40°C, which may have contributed to a report in one of the control tunnels (#4) of 200 bees clustering near the front of the hive. Additionally, because nominal treatment levels of afidopyropen and dimethoate were not verified analytically, there is uncertainty regarding actual exposure levels. However, measured residues in leaves, flowers, pollen and nectar indicate that bees were exposed to afidopyropen in the afidopyropen treatment groups; whereas, afidopyropen residues in the negative control were below the LOD of 0.03 mg ai/kg. Immediately (<4 h) following applications afidopyropen residues in *Phacelia* flowers and leaves were 3.34 ± 0.27 and 1.66 ± 0.18 mg a.i./kg, respectively; afidopyropen residues in flowers were significantly ($p<0.05$) higher than residues in leaves. M4401007 residues in flowers and leaves were 2.75 ± 0.16 and 3.30 ± 0.28 mg a.i./kg, respectively. Afidopyropen residues in pollen and nectar samples following applications (1 DAT) were 0.06 mg a.i./kg and <LOQ (0.006 mg a.i./kg), respectively; the photo-dimer M4401007I residues in pollen and nectar specimens were 0.08 mg/kg and <LOQ, respectively.

The study was consistent with OECD Guidance Document 75, and indicates that honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha exhibited significant adverse effects on adult worker bee mortality (-37.6%) and foraging activity (-12.3%). However, by 41 DAT, there were no statistical differences in numbers of adult, juveniles or brood or in the percentage of frame consisting of pollen and nectar in afidopyropen and negative control colonies. While there were statistically significant effects on adult bee mortality and foraging behavior in afidopyropen-treated colonies, these effects appear to be transient. Based on this study and the statistically significant effects on adult worker bee mortality and foraging activity, the NOAEL is <50 g a.i./ha.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

APPENDIX I. Output of Statistics Verified by the Reviewer

A. Summary Statistics & Tests

Adult Honeybee Mortality (no. dead bees/colony/d)

```
Call: lm(formula = value ~ group.trtmnt + group.phase, data = amort)

Residuals:
    Min      1Q  Median      3Q     Max
-141.84  -48.16   10.67   17.15  775.16

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)       93.980      9.264  10.145  < 2e-16 ***
group.trtmntref   68.865      8.379   8.218 1.39e-15 ***
group.trtmnttest   2.474      8.379   0.295 0.767914
group.phasemon  -104.125      8.913 -11.683  < 2e-16 ***
group.phasepre   -44.106     12.491  -3.531 0.000447 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 82.1 on 571 degrees of freedom
  (12 observations deleted due to missingness)
Multiple R-squared:  0.2931,  Adjusted R-squared:  0.2881
F-statistic: 59.18 on 4 and 571 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.5441, p-value < 2.2e-16

Bartlett test of homogeneity of variances
Bartlett's K-squared = 825.64, df = 2, p-value < 2.2e-16

Pre-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 7.2824, df = 2, p-value = 0.02622

Pairwise comparisons using Wilcoxon rank sum test

     cont  ref
ref  0.052 -
test 0.055 0.628

P value adjustment method: holm_

Exposure Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 63.505, df = 2, p-value = 1.622e-14

Pairwise comparisons using Wilcoxon rank sum test

     cont    ref
ref  2.2e-11 -
test 0.24    2.2e-11

P value adjustment method: holm_

Monitoring Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 51.622, df = 2, p-value = 6.173e-12

Pairwise comparisons using Wilcoxon rank sum test
```

```
     cont    ref
ref  7.5e-10 -
test 0.12    4.7e-07

P value adjustment method: holm

Overall Post-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 56.065, df = 2, p-value = 6.693e-13

Pairwise comparisons using Wilcoxon rank sum test

     cont    ref
ref  9.8e-13 -
test 0.01237 0.00022

P value adjustment method: holm

--------------------------------------------------------------------------------
Juvenile Honeybee Mortality (no. dead pupae/colony/d)

Call: lm(formula = value ~ group.trtmnt + group.phase, data = pmort)

Residuals:
    Min      1Q  Median      3Q     Max
-0.5903 -0.2885 -0.0541 -0.0437  6.7115

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.17535    0.07540   2.326 0.020392 *
group.trtmntref  0.23437    0.06820   3.436 0.000632 ***
group.trtmnttest -0.01042    0.06820  -0.153 0.878663
group.phasemon   -0.12121    0.07254  -1.671 0.095284 .
group.phasepre    0.18056    0.10167   1.776 0.076281 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.6682 on 571 degrees of freedom
  (12 observations deleted due to missingness)
Multiple R-squared:  0.04992, Adjusted R-squared:  0.04326
F-statistic:   7.5 on 4 and 571 DF,  p-value: 6.828e-06

Shapiro-Wilk normality test
W = 0.46804, p-value < 2.2e-16

Bartlett test of homogeneity of variances
Bartlett's K-squared = 185.23, df = 2, p-value < 2.2e-16

Pre-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 4.7506, df = 2, p-value = 0.09299

Exposure Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.10515, df = 2, p-value = 0.9488

Monitoring Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 16.723, df = 2, p-value = 0.0002337

Pairwise comparisons using Wilcoxon rank sum test
```

```
     cont    ref
ref  0.00055 -
test 0.86631 0.08968

P value adjustment method: holm

Overall Post-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 9.4521, df = 2, p-value = 0.008862

Pairwise comparisons using Wilcoxon rank sum test

     cont   ref
ref  0.0082 -
test 0.3245 0.3245

P value adjustment method: holm

--------------------------------------------------------------------------------
Colony Strength (no. adult bees/colony/d)
Call: lm(formula = value ~ trtmnt + dat, data = bsa)

Residuals:
    Min      1Q  Median      3Q     Max
-5129.9 -1346.5  -217.7  1343.5  7747.8

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  8336.59     567.08  14.701  < 2e-16 ***
trtmntref   -2589.17     656.98  -3.941 0.000194 ***
trtmnttest    -46.04     656.98  -0.070 0.944335
dat           119.69      17.90   6.686 5.19e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2276 on 68 degrees of freedom
Multiple R-squared:  0.4889,  Adjusted R-squared:  0.4664
F-statistic: 21.68 on 3 and 68 DF,  p-value: 5.787e-10

Shapiro-Wilk normality test
W = 0.97273, p-value = 0.1194

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.3447, df = 2, p-value = 0.8417

Bartlett test of homogeneity of variances
Bartlett's K-squared = 5.4724, df = 5, p-value = 0.361

-1 DAT
             Df   Sum Sq Mean Sq F value Pr(>F)
bsa_p1$trtmnt  2  8621817 4310908   1.276  0.325
Residuals      9 30411550 3379061

4 DAT
             Df   Sum Sq  Mean Sq F value  Pr(>F)
bsa_4$trtmnt  2 22596004 11298002   16.11 0.00106 **
Residuals     9  6310037   701115
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’

Simultaneous Tests for General Linear Hypotheses
```

```
Fit: aov(formula = bsa_4$n ~ bsa_4$trtmnt)

Linear Hypotheses:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept) == 0        7718.8      418.7  18.437  < 0.001 ***
bsa_4$trtmntref == 0   -2973.7      592.1  -5.023  0.00181 **
bsa_4$trtmnttest == 0   -130.0      592.1  -0.220  0.98920

11 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
bsa_11$trtmnt  2 43953379 21976690   3.836 0.0624 .
Residuals      9 51560844  5728983

22 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
bsa_22$trtmnt  2 35492817 17746408   4.844 0.0373 *
Residuals      9 32972956  3663662
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Simultaneous Tests for General Linear Hypotheses

Fit: aov(formula = bsa_22$n ~ bsa_22$trtmnt)

Linear Hypotheses:
                       Estimate Std. Error t value Pr(>|t|)
(Intercept) == 0        10416.3      957.0  10.884   <0.001 ***
bsa_22$trtmntref == 0   -3867.5     1353.5  -2.858    0.042 *
bsa_22$trtmnttest == 0   -487.5     1353.5  -0.360    0.957

32 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
bsa_32$trtmnt  2 42666163 21333081   4.009 0.0569 .
Residuals      9 47893544  5321505

41 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
bsa_41$trtmnt  2 20145504 10072752   2.276  0.159
Residuals      9 39836469  4426274
```

Colony Strength (no. juveniles/colony/d)

```
Call: lm(formula = value ~ trtmnt + dat, data = bsp)

Residuals:
    Min      1Q  Median      3Q     Max
-8074.8 -1969.9   346.5  2294.3  7874.4

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 13729.70     851.27  16.128  < 2e-16 ***
trtmntref   -3875.00     986.23  -3.929 0.000202 ***
trtmnttest  -1725.00     986.23  -1.749 0.084788 .
dat           -70.90      26.87  -2.638 0.010319 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 3416 on 68 degrees of freedom
Multiple R-squared:  0.2483,  Adjusted R-squared:  0.2151
F-statistic: 7.487 on 3 and 68 DF,  p-value: 0.0002108
Shapiro-Wilk normality test
W = 0.98215, p-value = 0.3994
```

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 5.6153, df = 2, p-value = 0.06035

Bartlett test of homogeneity of variances
Bartlett's K-squared = 7.879, df = 5, p-value = 0.163

-1 DAT
             Df   Sum Sq Mean Sq F value Pr(>F)
bsp_p1$trtmnt  2  1786667  893333   0.154   0.86
Residuals      9 52240000 5804444

4 DAT
             Df   Sum Sq  Mean Sq F value Pr(>F)
bsp_4$trtmnt  2 48980000 24490000   7.196 0.0136 *
Residuals     9 30630000  3403333
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Simultaneous Tests for General Linear Hypotheses

Fit: aov(formula = bsp_4$n ~ bsp_4$trtmnt)

Linear Hypotheses:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept) == 0       14700.0      922.4  15.937   <0.001 ***
bsp_4$trtmntref == 0   -4900.0     1304.5  -3.756   0.0103 *
bsp_4$trtmnttest == 0  -3050.0     1304.5  -2.338   0.0954 .

11 DAT
              Df    Sum Sq  Mean Sq F value   Pr(>F)
bsp_11$trtmnt  2 120126667 60063333   22.31 0.000325 ***
Residuals      9  24230000  2692222
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Simultaneous Tests for General Linear Hypotheses

Fit: aov(formula = bsp_11$n ~ bsp_11$trtmnt)

Linear Hypotheses:
                       Estimate Std. Error t value Pr(>|t|)
(Intercept) == 0         9750.0      820.4  11.884   <0.001 ***
bsp_11$trtmntref == 0   -7650.0     1160.2  -6.594   <0.001 ***
bsp_11$trtmnttest == 0  -2750.0     1160.2  -2.370   0.0907 .

22 DAT
              Df   Sum Sq  Mean Sq F value Pr(>F)
bsp_22$trtmnt  2 66046667 33023333   3.525  0.074 .
Residuals      9 84310000  9367778

32 DAT
              Df   Sum Sq  Mean Sq F value Pr(>F)
bsp_32$trtmnt  2 36086667 18043333   4.528 0.0436 *
Residuals      9 35860000  3984444
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Simultaneous Tests for General Linear Hypotheses

Fit: aov(formula = bsp_32$n ~ bsp_32$trtmnt)
```

```
Linear Hypotheses:
                        Estimate Std. Error t value Pr(>|t|)
(Intercept) == 0         13850.0      998.1  13.877   <0.001 ***
bsp_32$trtmntref == 0    -4200.0     1411.5  -2.976   0.0353 *
bsp_32$trtmnttest == 0   -1550.0     1411.5  -1.098   0.5387

41 DAT
              Df   Sum Sq Mean Sq F value Pr(>F)
bsp_41$trtmnt  2  7740000 3870000    1.22   0.34
Residuals      9 28540000 3171111
```

Foraging Activity (bees/m²/d)

```
Call: lm(formula = value ~ group.trtmnt + group.phase, data = forage.x)

Residuals:
    Min      1Q  Median      3Q     Max
-17.398  -5.964  -0.537   4.763  37.036

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)       17.3980     0.9251  18.807  < 2e-16 ***
group.trtmntref  -11.4341     1.2334  -9.270  < 2e-16 ***
group.trtmnttest  -1.8614     1.2334  -1.509    0.132
group.phasepre    -4.4842     1.1306  -3.966 9.45e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 8.182 on 260 degrees of freedom
Multiple R-squared:  0.3061,  Adjusted R-squared:  0.2981
F-statistic: 38.23 on 3 and 260 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.95952, p-value = 9.564e-07

Bartlett test of homogeneity of variances

Bartlett's K-squared = 0.89897, df = 2, p-value = 0.638

Pre-application Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.63153, df = 2, p-value = 0.7292

Exposure Phase
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 89.51, df = 2, p-value < 2.2e-16

Pairwise comparisons using Wilcoxon rank sum test

     cont    ref
ref  2.4e-16 -
test 0.03    3.1e-15

P value adjustment method: holm
```

Colony Condition - Brood (no. cells/colony/d as brood)

```
Call: lm(formula = value ~ trtmnt + dat, data = bcb)

Residuals:
    Min      1Q  Median      3Q     Max
-3652.4 -2648.6   448.8  1875.9  5523.9
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  2696.78     354.44   7.608 8.94e-13 ***
trtmntref    -709.51     410.64  -1.728   0.0855 .
trtmnttest    108.33     410.64   0.264   0.7922
dat            20.67      11.19   1.847   0.0661 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2464 on 212 degrees of freedom
Multiple R-squared:  0.03677, Adjusted R-squared:  0.02314
F-statistic: 2.697 on 3 and 212 DF,  p-value: 0.04684

Shapiro-Wilk normality test
W = 0.93609, p-value = 4.087e-08

Bartlett test of homogeneity of variances
Bartlett's K-squared = 1.0534, df = 2, p-value = 0.5905

Bartlett test of homogeneity of variances
Bartlett's K-squared = 2.5405, df = 5, p-value = 0.7704

-1 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.049127, df = 2, p-value = 0.9757

4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 3.7484, df = 2, p-value = 0.1535

11 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 2.0124, df = 2, p-value = 0.3656

22 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.23294, df = 2, p-value = 0.8901

32 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.0645, df = 2, p-value = 0.5873

41 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.69777, df = 2, p-value = 0.7055

-------------------------------------------------------------------------------
Colony Condition - Food (no. cells/colony/d as food)
Call: lm(formula = value ~ trtmnt + dat, data = bcf)

Residuals:
    Min      1Q  Median      3Q     Max
-7397.6 -3823.6  -763.1  3682.1 11602.4

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  7508.05     800.60   9.378  < 2e-16 ***
trtmntref    -383.33     927.52  -0.413  0.68003
trtmnttest     16.67     927.52   0.018  0.98569
dat           -72.92      25.27  -2.885  0.00453 **
---
```

```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4544 on 140 degrees of freedom
Multiple R-squared:  0.05764, Adjusted R-squared:  0.03744
F-statistic: 2.854 on 3 and 140 DF,  p-value: 0.03948

Shapiro-Wilk normality test
W = 0.95786, p-value = 0.0002176

Bartlett test of homogeneity of variances
Bartlett's K-squared = 1.6309, df = 2, p-value = 0.4424

Bartlett test of homogeneity of variances
Bartlett's K-squared = 19.23, df = 5, p-value = 0.001741

-1 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.071405, df = 2, p-value = 0.9649

4 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.0087729, df = 2, p-value = 0.9956

11 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.1956, df = 2, p-value = 0.9068

22 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 1.9658, df = 2, p-value = 0.3742

32 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.19602, df = 2, p-value = 0.9066

41 DAT
Kruskal-Wallis rank sum test
Kruskal-Wallis chi-squared = 0.30164, df = 2, p-value = 0.86

--------------------------------------------------------------------------------
Residue Levels (mg a.i./kg)

Source (flowers vs leaves) - Parent
Call: lm(formula = value.p ~ group, data = residues_test)

Residuals:
   Min     1Q Median     3Q    Max
-0.504 -0.397 -0.193  0.477  0.746

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   3.3440     0.2306   14.50 5.01e-07 ***
groupleaves  -1.6860     0.3261   -5.17 0.000853 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.5156 on 8 degrees of freedom
Multiple R-squared:  0.7696,  Adjusted R-squared:  0.7408
F-statistic: 26.73 on 1 and 8 DF,  p-value: 0.0008532

Shapiro-Wilk normality test
W = 0.86028, p-value = 0.07689
```

```
Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.68781, df = 1, p-value = 0.4069

Welch Two Sample t-test
t = 5.17, df = 6.8034, p-value = 0.001414
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval: 0.9103232 2.4616768

Source (flowers vs leaves) – M4401007
Call: lm(formula = value.m ~ group, data = residues_test)

Residuals:
   Min     1Q Median     3Q    Max
-0.854 -0.404  0.141  0.406  0.516

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   2.7540     0.2287   12.04 2.09e-06 ***
groupleaves   0.5500     0.3235    1.70    0.127
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.5114 on 8 degrees of freedom
Multiple R-squared:  0.2655,  Adjusted R-squared:  0.1736
F-statistic: 2.891 on 1 and 8 DF,  p-value: 0.1275

Shapiro-Wilk normality test
W = 0.89273, p-value = 0.182

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.94463, df = 1, p-value = 0.3311

Welch Two Sample t-test
t = -1.7003, df = 6.4866, p-value = 0.1363
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval: -1.3273274  0.2273274
```

APPENDIX II. Study Author's Summary of Observed Sublethal Behavioral Effects

Date [dd.mm.yyyy]	DAA	Replicate	Observation
24.06.2015	-1	C1	Bees were agressive
25.06.2015	0aa	T1	Up to 50 alive worker bees in DBT, coordination problems while moving
26.06.2015	1		Few[1)] worker bees falling from flowers while foraging
29.06.2015	4		Few[1)] worker bees falling from flowers while foraging
25.06.2015	0aa	T2	Up to 50 alive worker bees in the DBT, coordination problems while moving
26.06.2015	1		Few[1)] worker bees falling from flowers while foraging
25.06.2015	0aa	T3	Up to 60 alive worker bees in the DBT, coordination problems while moving
26.06.2015	1		Few[1)] worker bees falling from flowers while foraging, 10 to 20 alive worker bees in DBT shivering and cleaning
25.06.2015	0aa	T4	Up to 50 alive worker bees in DBT, coordination problems while moving
26.06.2015	1		Few[1)] worker bees falling from flowers while foraging
29.06.2015	4		Few[1)] worker bees falling from flowers while foraging
02.07.2015	7		Approximately 200 clustering in front of the hive and the DBT, probably due to high temperatures
25.06.2015	0aa	R1	Up to 60 worker bees in DBT and up to 30 worker bees on linen with coordination problems and/or cramping, foraging bees[1)] showing intoxication symptoms like problems while landing or with the nectar uptake
26.06.2015	1		Approximately 20 worker bees in DBT with coordination problems and/or cramping
30.06.2015	5		7 cramping worker bees in DBT
01.07.2015	6		12 cramping worker bees in DBT
02.07.2015	7		Worker bees clustering at the outside of the hive, 1 drone with deformed wings
25.06.2015	0aa	R2	Up to 60 worker bees in DBT and up to 50 worker bees on linen with coordination problems and/or cramping, foraging bees[1)] showing intoxication symptoms like problems while landing or with the nectar uptake, falling on the floor while flying
26.06.2015	1		Approximately 30 worker bees in DBT with coordination problems and/or cramping
30.06.2015	5		3 cramping worker bees in DBT
01.07.2015	6		8 cramping worker bees in DBT
02.07.2015	7		7 cramping worker bees in DBT

DAA = days after application; T = test item group; R = reference item group; aa = after application; DBT = dead bee trap; [1)] the number of bees was not recorded

Date [dd.mm.yyyy]	DAA	Replicate	Observation
25.06.2015	0aa	R3	Up to 50 worker bees in DBT and up to 50 worker bees on linen with coordination problems and/or cramping, foraging bees[1)] showing intoxication symptoms like problems while landing or with the nectar uptake, falling on the floor while flying
26.06.2015	1		Approximately 10 worker bees in DBT with coordination problems and/or cramping
30.06.2015	5		8 cramping worker bees, partly with coordination problems, in DBT
01.07.2015	6		4 cramping worker bees, partly with coordination problems, in DBT
02.07.2015	7		11 cramping worker bees, partly with coordination problems, in DBT
25.06.2015	0aa	R4	Up to 54 worker bees in DBT and up to 10 worker bees on linen with coordination problems and/or cramping, foraging bees[1)] showing intoxication symptoms like problems while landing or with the nectar uptake, falling on the floor while flying
26.06.2015	1		Approximately 30 worker bees in DBT with coordination problems and/or cramping

DAA = days after application; T = test item group; R = reference item group; aa = after application; DBT = dead bee trap; [1)] the number of bees was not recorded